Version with Markings to Show Changes Made

Please amend the specification as follows:

IN THE SPECIFICATION

Please amend the paragraph on page 1, lines 7-9, as follows:

--This application claims priority to U.S. [Provisinal] **Provisional** Application No. 60/241,488, filed on October 18, 2000, U.S. [Provisinal] **Provisional** Application No. 60/256,067 filed on December 15, 2000 and U.S. [Provisinal] **Provisional** Application No. 60/282,156, filed on April 6, 2001.

Please amend the paragraph on page 2, lines 1-8, as follows:

--"Real-time PCR" refers to a polymerase chain reaction that is monitored, usually by fluorescence, over time during the amplification process, to measure a parameter related to the extent of amplification of a particular sequence, such as the extent of hybridization of a probe to amplified target sequences. The DNA generated within a PCR is detected on a cycle by cycle basis during the PCR reaction. The amount of DNA increases faster the more template sequences are present in the original sample. When enough amplification products are made a [threshhold] **threshold** is reached at which the PCR products are detected. Thus amplification and detection are performed simultaneously in the same tube.

Please amend the paragraph on page 4, lines 12-22, as follows:

Quantitation ([indicationg] **indicating** how many copies of the target nucleic acid are present) has primarily been applied to chronic viral infections, especially hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections. The level of viremia has prognostic implications, and has been used to demonstrate response to antiviral drugs. PCR is quite sensitive, but it is not inherently quantitative. The amount of the final PCR product is usually similar from an initial sample containing 10 or 10,000 copies. This limitation can be overcome by serial dilution of the clinical sample until no target DNA is detected, or by the addition of synthetic competitor DNA molecules. The competitor molecules have regions complementary to the two primers, but differ in some way from the natural target (e.g., a different length). By comparing the amount of the natural and competitor PCR products, a rough estimation of the number of target molecules in the sample is possible.

Please amend the paragraph on page 8, lines 1-6, as follows:

--WO 00/44936 filed by Bavarian Nordic Research Institute A/S describes a real-time PCR method for the detection and quantification of variants of nucleic acid sequences that differ in the probe-binding site. The method is based in the complete or partial amplification of the same region of the variants and the addition of two or more [oligonucleoitde] **oligonucleotide**

probes to the same PCR mixture, each probe being specific for the probe-binding site of at least one variant.

Please amend the paragraph on page 13, lines 10-17, as follows:

--Processes and methods for the simultaneous quantification of nucleic acids in diseased cells that are based on real-time PCR are provided. The real-time-PCR protocol is an excellent tool for reliable quantification of *in vitro* drug screening and evaluation protocols to determine the efficacy of potential anti-viral agents. Quantification using these [simulateous] **simultaneous** PCR cycle threshold (Ct) detection techniques during one-step real-time RT-PCR (Applied Biosystems, CA) eliminates the variability resulting from quantification of end-point RT-PCR products. In addition, the mitochondrial toxicity assay is an added tool to assess potential side-effects for these chemotherapeutic agents.

Please amend the paragraph on page 14, lines 10-18, as follows:

--With the availability of both the viral ΔCt data and the host ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting the host ΔCt value from the viral ΔCt value. This results in ΔΔCt values; a value above 0 means that there is more inhibitory effect on the viral nucleic acid, a ΔΔCt value below 0 means that the host nucleic acid is more affected. As a general rule, ΔΔCt values above 2 are considered as significantly different from the no-drug treatment control, and hence, exhibits useful antiviral activity. However, compounds with a ΔΔCt value of less than 2, but showing limited molecular [cytotoxicty] **cytotoxicity** data (rRNA [ΔCT] **ΔCt** between 0 and 2) may also be desired for certain applications requiring compounds with low toxicity.

Please amend the paragraph on page 14, lines 19-31, as follows:

--As an example, a compound might reduce the host RNA polymerase activity, but not the host DNA polymerase activity. Therefore, quantification of rDNA or β-actin DNA (or any other host DNA fragment) and comparison with DNA levels of the no-drug control is a relative measurement of the inhibitory effect of the test compound on cellular DNA polymerases. With the availability of both the HCV ΔCt data and the rDNA ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting both ΔCt values from each other. This results in ΔΔCt values; a value above 0 means that there is more inhibitory effect on the viral encoded polymerase, a ΔΔCt value below 0 means that the host rDNA levels are more affected than the viral nucleic acid levels. As a general rule, ΔΔCt values above 2 are considered as significantly different from the no-drug treatment control, and hence, is an interested compound for further evaluation. However, compounds with a ΔΔCt value of less than 2, but with limited molecular [cytotoxicty] **cytotoxicity** (rDNA [ΔCT] **ΔCt** between 0 and 2) are also possible active candidate compounds for further evaluation

Please amend the paragraph on page 15, lines 9-14, as follows:

--In a particular embodiment, the level of transcription of the viral and host nucleic acid is compared to that of a standard, including but not limited to, a known viral infected host cell,

or alternatively, an internal standard can be established by comparing the extent of transcription of the host and viral nucleic acid over a number of samples from the host to monitor and measure the change in infection. In another embodiment, the data can be assessed as described above through the use of [ΔCT] **ΔCt** and ΔΔCt values.

Please amend the paragraph on page 17, lines 14-15, as follows:

--In a particular embodiment of the present invention, process of [simulatneous] **simultaneous** real-time PCR includes the following steps:

Please amend the paragraphs on page 17, lines 22-25, as follows:

iii) wherein at least one independently labeled oligonucleotide or probe that [hydrbiridizes] **hybridizes** to a target viral nucleic acid sequence; and

iv) at least one independently labeled oligonucleotide or probe that [hydrbiridizes] **hybridizes** to a target host nucleic acid sequence;

Please amend the paragraph on pages 20-21, lines 24-29 and 1-2, as follows:

--Processes and methods for the simultaneous quantification of nucleic acids in diseased cells that are based on real-time PCR are provided. The real-time-PCR protocol is an excellent tool for reliable quantification of *in vitro* drug screening and evaluation protocols to determine the efficacy of potential anti-viral agents. Quantification using these [simulateous] **simultaneous** PCR cycle threshold (Ct) detection techniques during one-step real-time RT-PCR (Applied Biosystems, CA) eliminated the variability resulting from quantification of end-point RT-PCR products. In addition, the mitochondrial toxicity assay is an added tool to assess potential side-effects for these chemotherapeutic agents.

Please amend the paragraph on page 21, lines 22-30, as follows:

--With the availability of both the viral ΔCt data and the host ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting the host ΔCt value from the viral ΔCt value. This results in ΔΔCt values; a value above 0 means that there is more inhibitory effect on the viral nucleic acid, a ΔΔCt value below 0 means that the host nucleic acid is more affected. As a general rule, ΔΔCt values above 2 are considered as significantly different from the no-drug treatment control, and hence, exhibits useful antiviral activity. However, compounds with a ΔΔCt value of less than 2, but showing limited molecular [cytotoxicty] **cytotoxicity** data (rRNA ΔCT between 0 and 2) may also be desired for certain applications requiring compounds with low toxicity.

Please amend the paragraph on page 22, lines 1-13, as follows:

--As an example, a compound might reduce the host RNA polymerase activity, but not the host DNA polymerase activity. Therefore, quantification of rDNA or β-actin DNA (or any

other host DNA fragment) and comparison with DNA levels of the no-drug control is a relative measurement of the inhibitory effect of the test compound on cellular DNA polymerases. With the availability of both the HCV ΔCt data and the rDNA ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting both ΔCt values from each other. This results in ΔΔCt values; a value above 0 means that there is more inhibitory effect on the viral encoded polymerase, a ΔΔCt value below 0 means that the host rDNA levels are more affected than the viral nucleic acid levels. As a general rule, ΔΔCt values above 2 are considered as significantly different from the no-drug treatment control, and hence, is an interested compound for further evaluation. However, compounds with a ΔΔCt value of less than 2, but with limited molecular [cytotoxicty] **cytotoxicity** (rDNA [ΔCT] **ΔCt** between 0 and 2) are also possible active candidate compounds for further evaluation

Please amend the paragraph on page 22, lines 22-27, as follows:

--In a particular embodiment, the level of transcription of the viral and host nucleic acid is compared to that of a standard, including but not limited to, a known viral infected host cell, or alternatively, an internal standard can be established by comparing the extent of transcription of the host and viral nucleic acid over a number of samples from the host to monitor and measure the change in infection. In another embodiment, the data can be assessed as described above through the use of [ΔCT] **ΔCt** and ΔΔCt values.

Please amend the paragraph on page 25, lines 1-2, as follows:

--In a particular embodiment of the present invention, a method of [simulatneous] **simultaneous** real-time PCR includes the following steps:

Please amend the paragraphs on page 25, lines 9-12, as follows:

iii) wherein at least one independently labeled oligonucleotide or probe that [hydrbiridizes] **hybridizes** to a target viral nucleic acid sequence; and

iv) at least one independently labeled oligonucleotide or probe that [hydrbiridizes] **hybridizes** to a target host nucleic acid sequence;

Please amend the paragraph on page 33, lines 19-25, as follows:

--In a preferred embodiment of the invention, the oligonucleotides used to amplify β-actin (primers) are [sense] sens 5'-GCGCGGCTACAGCTTCA-3' (Sequence ID No. 1) and antisense 5'-TCTCCTTAATGTCACGCACGAT-3' (Sequence ID No. 2). The labeled [oligonulceotide] **oligonucleotide** (probe) used to detect host nucleic acid has a sequence of 5'-CACCACGGCCGAGCGGGA-3' (Sequence ID No. 3). In one [emobidment] **embodiment**, the probe is labeled with a reporter at the 5'-end and a quencher molecule at the 3'-end, and in particular, the reporter, FAM, at the 5' end, and the quencher molecule, TAMRA, at the 3' end.

Please amend the paragraph on page 34, lines 1-9, as follows:

[Mitochondiral] **Mitochondrial** Nucleic Acid

In one embodiment, the mitochondrial nucleic acids can be specifically derived from mitochondrial DNA. In an alternate embodiment, the [mitochondiral] **mitochondrial** nucleic acids can be specifically derived from mitochondrial RNA. In an alternate embodiment, the [mitochondiral] **mitochondrial** nucleic acids are complementary to sequences from [themitochondrial] **the mitochondrial** COXII gene. Any suitable primers and/or probes can be used. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to TaqMan, molecular beacons, single dye probe, SYBR green, Amplifluor probes and dual labeled probe sets.

Please amend the paragraph on page 34, lines 10-16, as follows:

-- In a preferred embodiment of the invention, the oligonucleotides used to amplify mitochondrial nucleic acids (primers) are sense [sense sense] 5'-TGCCCGCCATCATCCTA-3' (Sequence ID No. [19] **4**) and 5'-TCGTCTGTTATGTAAAGGATGCGT-3' (Sequence ID No. [20] **5**). The labeled [oligonulceotide] **oligonucleotide** (probe) used to detect host nucleic acid has a sequence of 5'-TCCTCATCGCCCTCCCATCCC-3' (Sequence ID No. [21] **6**). In one [emobidment] **embodiment**, the probe is labeled with a reporter at the 5'-end and a quencher molecule at the 3'-end, and in particular, the reporter, TET, at the 5' end, and the quencher molecule, TAMRA, at the 3' end.--

Please amend the paragraph on page 35, lines 16-23, as follows:

--In a preferred embodiment of the invention, the oligonucleotides used to amplify HIV-1 (primers) are sense 5'–TGGGTTATGAACTCCATCCTGAT-3' (Sequence ID No. [4] **7**) and antisense 5'–TGTCATTGACAGTCCAGCTGTCT-3' (Sequence ID No. [5] **8**). The labeled [oligonulceotide] **oligonucleotide** (probe) used to detect HIV-1 viral load has a sequence of 5'-TTTCTGGCAGCTCTCGGCTGTACTGTCCATT-3' (Sequence ID No. [6] **9**). In one [emobidment] **embodiment**, the probe is labeled with a reporter at the 5'-end and a quencher molecule at the 3'-end, and in particular, the reporter, FAM, at the 5' end, and the quencher molecule, TAMRA, at the 3' end.--

Please amend the paragraph on pages 35-36, lines 25-28 and 1-4, as follows:

--In another embodiment of the invention, the target viral nucleic acid is from HCV. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are derived from [thighly] **highly** conserved sequences complementary to the RNA sequences present in HCV, such as the HCV 5' non-coding region. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to TaqMan, molecular beacons, single dye probe, SYBR green, Amplifluor probes and dual labeled probe sets.

Please amend the paragraph on page 36, lines 5-11, as follows:

--In a preferred embodiment of the invention, the oligonucleotides used to amplify HCV (primers) are sense 5'-AGCCATGGCGTTAGTA(T/A)GAGTGT-3' (Sequence ID No. [7] **10**) and antisense 5'-TTCCGCAGACCACTATGG-3' (Sequence ID No. [8] **11**). The labeled [oligonulceotide] **oligonucleotide** (probe) used to detect HCV viral load has a sequence of 5'-CCTCCAGGACCCCCCTCCC-3' (Sequence ID No. [9] **12**). In one [emobidment] **embodiment**, the probe is labeled with a reporter at the 5'-end and a quencher molecule at the 3'-end, and in particular, the reporter, FAM, at the 5' end, and the quencher molecule, TAMRA, at the 3' end.--

Please amend the paragraph on page 36, lines 13-20, as follows:

--In another embodiment of the invention, the target viral nucleic acid is from BVDV. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are derived from [thighly] **highly** conserved sequences complementary, such as sequences complementary to nucleotides 1611 to 1751 of the NS5B gene. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to TaqMan, molecular beacons, single dye probe, SYBR green, Amplifluor probes and dual labeled probe sets.

Please amend the paragraph on page 36, lines 21-28, as follows:

--In a preferred embodiment of the invention, the oligonucleotides used to amplify BVDV (primers) are sense [sense] 5'-AGTCTTCAGTTTCTTGCTGATGT-3' (Sequence ID No. [10] **13**) and antisense 5'-TGTTGCGAAAGGACCAACAG-3' (Sequence ID No. [11] **14**). The labeled [oligonulceotide] **oligonucleotide** (probe) used to detect BVDV viral load has a sequence of 5'-AAATCCTCCTAACAAGCGGGTTCCAGG-3' (Sequence ID No. [12] **15**). In one [emobidment] **embodiment**, the probe is labeled with a reporter at the 5'-end and a quencher molecule at the 3'-end, and in particular, the reporter, FAM, at the 5' end, and the quencher molecule, TAMRA, at the 3' end.--

Please amend the paragraph on page 37, lines 2-9, as follows:

--In another embodiment of the invention, the target viral nucleic acid is from HBV. Any suitable primers and/or probes can be used. In a specific embodiment of the present invention, the primers and/or probes are derived from [thighly] **highly** conserved sequences complementary to the DNA sequences present in HBV, such as the amino-terminal region of the HBV surface antigen gene. The probe comprises a reporter and quencher that provides a detectable signal upon amplification. Any reporter/quencher probe set can be used, including, but not limited to TaqMan, molecular beacons, single dye probe, SYBR green, Amplifluor probes and dual labeled probe sets.

Please amend the paragraph on page 37, lines 10-17, as follows:

--In a preferred embodiment of the invention, the oligonucleotides used to amplify HBV (primers) are sense 5'-GGACCCCTGCTCGTGTTACA-3' (Sequence ID No. [13] **16**) and antisense 5'-GAGAGAAGTCCACCACGAGTCTAG-3' (Sequence ID No. [14] **17**). The labeled [oligonulceotide] **oligonucleotide** (probe) used to detect HBV viral load has a sequence of 5'-TGTTGACAA(A/G)TCCTCACAATACC(A/G)CAGA-3' (Sequence ID No. [15] **18**). In one [emobidment] **embodiment**, the probe is labeled with a reporter at the 5'-end and a quencher molecule at the 3'-end, and in particular, the reporter, FAM, at the 5' end, and the quencher molecule, TAMRA, at the 3' end.--

Please amend the paragraph on pages 37-38, lines 27-29 and 1-5, as follows:

--In a preferred embodiment of the invention, the oligonucleotides used to amplify RSV (primers) are sense sense 5'-CAACAACCCTAATCATGTGGTATCA-3' (Sequence ID No. [16] **19**) and antisense 5' -CCGGTTGCATTGCAAACA-3' (Sequence ID No. [17] **20**). The labeled [oligonulceotide] **oligonucleotide** (probe) used to detect RSV viral load has a sequence of 5'-TGACAGGCAAAGAAAGAGAACTCAGTGTAGGTAGA-3' (Sequence ID No. [18] **21**). In one [emobidment] **embodiment**, the probe is labeled with a reporter at the 5'-end and a quencher molecule at the 3'-end, and in particular, the reporter, FAM, at the 5' end, and the quencher molecule, TAMRA, at the 3' end.--

Please amend the paragraph on page 41, lines 3-9, as follows:

--Reagent kits that support quantitative amplification and detection in multiplex are commercially available. The QPCR kits are used with DNA templates, either to detect DNA mutations or to measure gene or viral copy number. The QRT-PCR kits are used with RNA templates, typically for measuring RNA levels. Mutations can also be detected in expressed RNA with these kits. These kits have the capability of high performance with various fluorescent detection systems, including, the AmpliFluor system, molecular beacons, TaqMan® probes, dual fluorophore approach, single-dye primers and DNA [bynding] **binding** dyes.

Please amend the paragraph on page 49, lines 25-27, as follows:

--In particular, the kit comprises a primer/probe set for viral nucleic acid for HIV-1 wherein the primers are given by Sequence ID No. [4] **7** and [5] **8**, and the probe is a sequence given by Sequence ID No. [6] **9** along with a fluorescent dye and quenching dye.--

Please amend the paragraphs on page 50, lines 1-15, as follows:

--In particular, the kit comprises a primer/probe set for viral nucleic acid for HCV wherein the primers are given by Sequence ID No. [7] **10** and [8] **11**, and the probe is a sequence given by Sequence ID No. [9] **12** along with a fluorescent dye and quenching dye.

In particular, the kit comprises a primer/probe set for viral nucleic acid for BVDV wherein the primers are given by Sequence ID No. [10] **13** and [11] **14**, and the probe is a sequence given by Sequence ID No. [12] **15** along with a fluorescent dye and quenching dye.

In particular, the kit comprises a primer/probe set for viral nucleic acid for HBV wherein the primers are given by Sequence ID No. [13] **16** and [14] **17**, and the probe is a sequence given by Sequence ID No. [5] **8** along with a fluorescent dye and quenching dye.

In particular, the kit comprises a primer/probe set for viral nucleic acid for RSV wherein the primers are given by Sequence ID No. [16] **19** and [17] **20**, and the probe is a sequence given by Sequence ID No. [18] **21** along with a fluorescent dye and quenching dye.

In particular, the kit comprises a primer/probe set for host [mitochondiral] **mitochondrial** nucleic acid wherein the primers are given by Sequence ID No. [19] **4** and [20] **5**, and the probe is a sequence given by Sequence ID No. [21] **6** along with a fluorescent dye and quenching dye.

Please amend the paragraph on pages 51-52, lines 22-23 and 1-11, as follows:

--The TaqMan probe and primers were designed by using the Primer Express software (Applied Biosystems, CA) and are covering highly conserved sequences complementary to the DNA sequences present in HIV-1 RNA. By scanning the different genotypes of group M for regions containing only minor variability, the conserved domain was discovered. As a result, the region in the HIV-1 RT domain between codon 200 and 280 fulfilled the required criteria; thus this region was used to design an appropriate set of primers and probe that could work in real time PCR ("RT-PCR"). Primer sequences are as follows: sense 5'-TGGGTTATGAACTCCATCCTGAT-3' (Sequence ID No. [ ] **7**) and 5'-TGTCATTGACAGTCCAGCTGTCT-3' (Sequence ID No. [ ] **8**); the probe sequence is 5'-fluoresent dye-TTTCTGGCAGCACTATAGGCTGTACTGTCCATT-quenching dye-3' (Sequence ID No. [ ] **22**). In this particular case, the probe was labeled with FAM at the 5' end, and the quencher molecule is TAMARA, provided at the 3' end. Any other combination of reporter and quencher dyes can be used as well.--

Please amend the paragraph on page 60, Lines 14-22, as follows:

--HBV viral particles are released from at leasts three different cell lines: HepG2.2.1.5, HEPAD38 and HepAD79 cell lines. The cells were brought into culture for several days and total nucleic acids present in the culture supernatant, or in the cells, was prepared using commercially available columns (QIAamp Viral RNA mini Kit, Qiagen, CA). PCR–amplified DNA was detected in real-time by monitoring increases in fluorescence signal. A total of 5 µL DNA was RT-amplified using reagents and conditions as described by the manufacturer (Applied Biosystems, CA). The standard curve ranged from 2 copies to over 2×10^7 copies per reaction mix. Copy numbers were calculated [form] **from** OD260 values obtained from an HBV standard. Correlation coefficient is in all experiments greater than 0.99.

Please amend the paragraph on page 61, Lines 3-12, as follows:

--BVDV viral particles are released from infection experiments using the strain NADL on MDBK cells (both available [form] **from** ATTC). After infection, the cell were brought into culture for several days and total nucleic acids present in the culture supernatant, or in the cells, was prepared using commercially available columns (QIAamp Viral RNA mini Kit, Qiagen, CA). RT-PCR–amplified RNA was detected in real-time by monitoring increases in fluorescence signal. A total of 5 µL DNA was RT-amplified using reagents and conditions as described by the manufacturer (Applied Biosystems, CA). The standard curve ranged from 0.6

plaque forming units to over 6 x 10^3 plaque forming units per reaction mix. Plaque forming units were calculated form traditional plaque assays. Correlation coefficient is in all experiments greater than 0.99.

Please amend the paragraph on pages 63-64, lines 26-30 and 1-9, as follows:

--Total DNA is isolated from cultured HepG2 cells by commercially available columns (QIAamp DNA Blood Mini Kit, Qiagen, CA). Total DNA was eluted from columns in 200 μL of water. The mitochondrial gene and nuclear gene are then amplified with a quantitative real-time PCR protocol using suitable primers and probes. A set of primers and fluorescent probes for both nuclear and mitochondrial DNA or RNA was designed; the endogenous control DNA primer set is given by 5'-GCG CGG CTA CAG CTT CA-3' (Sequence ID [ ] No. **1**) and 5'-TCT CCT TAA TGT CAC GCA CGA T-3' (Sequence ID No. [ ] **23**); the mitochondrial DNA primer set is given by 5'-TGC CCG CCA TCA TCC TA-3' (Sequence ID No. [ ] **4**) and 5'-TCG TCT GTT ATG TAA AGG ATG CGT-3' (Sequence ID No. [ ] **5**). The probe for nuclear gene is given by 5'-fluorescent Dye-CAC CAC GGC CGA GCG GGA-fluorescent quencher-3' (Sequence ID No. [ ] **24**); fluorescent labeled probes for mitochondrial genome is given by 5'-fluorescent Dye-TCC TCA TCG CCC TCC CAT CCC-fluorescent quencher-3' (Sequence ID No. [ ] **25**). Reagents and conditions used in quantitative PCR were purchased from PE-Applied Biosystems.--

Please amend the paragraph on page 66, Lines 1-11, as follows:

--With the availability of both the HCV ΔCt data and the rRNA ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting both ΔCt values from each other. This results in ΔΔCt values; a value above 0 means that there is more inhibitory effect on the replicon encoded polymerase, a ΔΔCt value below 0 means that the host rRNA levels are more affected than the replicon levels. As an illustration of this technology, the antiviral activity of tested compounds, expressed as ΔΔCt values, is given in **Figure 5**. As a general rule, ΔΔCt values above 2 are considered as significantly different from the no-drug treatment control, and hence, is an interested compound for further evaluation. However, compounds with a ΔΔCt value of less than 2, but showing limited molecular [cytotoxicty] **cytotoxicity** data (rRNA [ΔCT] **ΔCt** between 0 and 2) are also possible active candidate compounds for further evaluation

Please amend the paragraph on page 66, Lines 12-24, as follows:

--In another typical setting, a compound might reduce the host RNA polymerase activity, but not the host DNA polymerase activity. Therefore, quantification of rDNA or beta-actin DNA (or any other host DNA fragment) and comparison with DNA levels of the no-drug control is a relative measurement of the inhibitory effect of the test compound on cellular DNA polymerases. With the availability of both the HCV ΔCt data and the rDNA ΔCt, a specificity parameter can be introduced. This parameter is obtained by subtracting both ΔCt values from each other. This results in ΔΔCt values; a value above 0 means that there is more inhibitory effect on the replicon encoded polymerase, a ΔΔCt value below 0 means that the host rDNA levels are more affected than the replicon levels. As a general rule, ΔΔCt values above 2 are considered as significantly different from the no-drug treatment control, and hence, is an interested compound for further

evaluation. However, compounds with a ΔΔCt value of less than 2, but with limited molecular cytotoxicty (rDNA [ΔCt] **ΔCt** between 0 and 2) are also possible active candidate compounds for further evaluation[].

Please amend the paragraphs on pages 72-73, lines 17-25 and 1-14, as follows:

--As one illustration of this method, in the case of BVDV in MDBK cells, in a first step, viral RNA is isolated from 140 μL of the cell culture supernatant by means of a commercially available column (Viral RNA extraction kit, QiaGen, CA). The viral RNA is then eluted from the column to yield a total volume of 60 μL, and subsequently amplified with a quantitative RT-PCR protocol using a suitable primer for the BVDV NADL strain. A quenched fluorescent probe molecule is hybridized to the BVDV DNA, which then undergoes exonucleolytic degradation resulting in a detectable fluorescent signal. Therefore, the RT-PCR amplified DNA was detected in real time by monitoring the presence of fluorescence signals. The TaqMan probe molecule 5'-6-FAM-AAATCCTCCTAACAAGCGGGTTCCAGG-TAMRA 3' (Sequence ID No [ ] **26**) and primers sense: 5'-AGCCTTCAGTTTCTTGCTGATGT-3' (Sequence ID No [ ] **27**); and antisense: 5'–TGTTGCGAAAGCACCAACAG-3' (Sequence ID No [ ] **28**) were designed with the aid of the Primer Express software (PE-Applied Biosystems) to be complementary to the BVDV NADL NS5B region. A total of 10 μL of RNA was analyzed in a 50 μL RT-PCR mixture. Reagents and conditions used in quantitative PCR were purchased from PE-Applied Biosystems. The standard curve that was created using the undiluted inoculum virus ranged from 6000 plaque forming units (PFU) to 0.6 PFU per RT-PCR mixture. A linear range of over 4-logs was routinely obtained.

A comparable approach can be taken to measure the amount of other Flaviviridae (more importantly HCV, YFV, Dengue, West Nile Virus and others) in a clinical sample or in a tissue culture sample. For example, the combination of HCV RNA purification with real-time RT-PCR using the following primers (5'-TTCCGCAGACCACTATGG-3' (Sequence ID No. [ ] **11**) and 5'-AGCCATGGCGTTAGTATGAGTGT-3' (Sequence ID No. [ ] **29**) and probe (5'-6-FAM-CCTCCAGGACCCCCCCTCCC-TAMRA-3' (Sequence ID No. [ ] **30**) resulted in a 7-log linear range of viral load detection.--